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Induction and spread of antibiotic resistance in livestock

Objectives Residues of veterinary antibiotics are regularly detected in manure, soil and water. But the fate and behaviour of these antibiotics in the environment is not yet clear. Hence, there is limited information available on the potential risk of these substances to the health of humans and animals or on possible ecological effects. This project aimed at improving knowledge regarding the environmental fate of antibiotics by quantifying the fluxes from manure to soils and water bodies.

Conclusions When the project was started, little quantitative use data was available for veterinary antibiotics in Switzerland. A feasibility study was carried out (Spring et al, 2003, "Flow of antibiotics in Swiss livestock production") on possible approaches to obtaining use data under Swiss (legal) conditions. In the meantime, several of the resulting suggestions have been implemented, and more precise data are available on the use of veterinary antibiotics in Swiss agriculture.
The field experiments that were carried out showed that veterinary antibiotics like sulfonamides (SA) may reach agricultural soils in amounts comparable to herbicide application rates and may persist in soils for several weeks or months (even exceeding 100 ng/g soil, corresponding to the trigger value in guidelines for the registration of new veterinary pharmaceuticals above which further investigations are required). Manure strongly influences the fate and transport of SA by increasing their chemical availability for transport and the runoff volume. However, the concentrations in the soil solution decreased rapidly, so that highest SA concentrations in the surface waters were measured during the first rain event after manure application. SA residues could also be detected in lake and groundwater in very low concentrations (about 0.5–1 ng/L). The relevance of such trace concentrations remains an open question.
The findings from the field demonstrating that the mobility of SA for transport in the soil is strongly influenced by contact time and by pH were corroborated in laboratory experiments. The experiments further revealed the importance of organic carbon in soils for the sorption of SA. Therefore, risk assessment for veterinary antibiotics has to consider not only substance properties but also the physical and chemical influence of the application matrix – manure or excrements – and of the soils.
Finally, it could be shown that not only antibiotics may be spread with manure but large amounts of resistance genes as well. This transfer might be more important for the spread and maintenance of antibiotic resistance than the transport of the antibiotics themselves. However, the data also showed that agricultural soils may be a considerable reservoir of resistance genes even without input of manure that contains antibiotics. In general, the outcomes of this project indicate a need for more prudent and judicious use of antibiotics.

Main results and findings

Flow of antibiotics in Swiss livestock production In the feasibility study (Spring et al, 2003, "Flow of antibiotics in Swiss livestock production"), some specifics for obtaining more precise and reliable data on the use of veterinary antibiotics in Swiss farming were proposed. Since then, several of the proposals have been implemented, and more precise data on the use of veterinary antibiotics are available through:
– Swissmedic, which provided detailed use data for 2004 for the different compound classes.
– Farming records, because the legal situation has changed ("Tierarzneimittelverordnung, TAMV", in force since January 2004), and farmers have to report not only medication but also the administered dose of antibiotics. Unfortunately, research generally has no access to these data.

Experimental mass flux analysis of sulfonamides (SA) After their use in agriculture, SA reach the soil through manure. New analytical procedures were needed and developed to quantify sulfonamides fluxes from manure to soils and water bodies. The methods allow measurements of many (hundreds) of samples, reliable quantification to the low ng/L range and take into account what is called "aging" of substances in soils, namely, the contact time, which affects extraction efficiencies. It was also shown that extraction efficiencies were dependent on the extraction temperature (an effect that has been completely overlooked so far, so that data on the persistence of SA are underestimated in previous studies). The main outcomes of the studies on fate and transport of veterinary SA can be summarised as follows:

- The manure has a strong influence on the fate and transport of SA. It not only increased the chemical availability of the antibiotics in soils for transport but the runoff volume as well. This resulted in 10 to 40 times larger losses from manured plots compared to the aqueous controls (with losses between 0.2% and 2% of the applied mass). These effects would not have been predictable, if only interactions between the soil and the SA had been examined.
- Field-scale experiments and monitoring studies at the scale of small catchments, including tributaries of Lake Sempach (a region characterised by intensive animal husbandry), and in ground and lake water demonstrated that:
  - SA are lost to surface waters during a few discharge events causing high SA concentrations for relatively short periods.
  - SA residues can be detected in lake and groundwater in very low concentrations (about 0.5–1 ng/L). The relevance of such trace concentrations remains an open question.
- SA residues may reach agricultural soils in amounts comparable to herbicide application rates (tens to several hundred g of antibiotics per ha and yr). Residues were quite persistent, even exceeding 100 ng/g soil – a value corresponding to the trigger value in the guidelines for registration of new veterinary pharmaceuticals above which further investigations are required – over more than 3 months.
- Crucial factors controlling the mobility of SA for transport in the soil are contact time, pH (SA are present as cations, neutral molecule or anion within the pH range found in agricultural soils) and the content of organic carbon of a soil.

Therefore, risk assessment for veterinary antibiotics has to consider not only substance properties but also the physical and chemical influence of the application matrix – manure or excrements – and the receiving soils.

Biomimetic measures of bio-availability of SA in soils The persistence of SA in soils raised the question about their bioavailability. A new methodolgy was sought to directly measure this quantity by quantifying products of the biochemical pathways disturbed by the SA. In bacteria, SA interfere with the biosynthesis of folic acid forming dihydropterin-sulfonamides (DH-SA). The results showed that dihydrofolic acid (DHF, a structural analogon to DH-SA) is not stable outside the organisms. The excreted pterin-SA, however, could be used as proxy for the bio-availability of SA. Further experiments (i.e. measurement of uptake of sulfathiazole by bacteria under different conditions, sorption and desorption processes, uptake kinetic of sorbed SA) are ongoing. Interestingly, the DH-SA set free by a bacterial cell may be cleaved into p-ABG (p-aminobenzoylglutamic acid) or, more important, into the structural analogous, which is the original SA, suggesting a kind of recycling of SA once removed from the organism.

Relevance for antibiotic resistance The manure used in the study contained a high diversity of resistance genes (i.e. resistance against tetracyclines and sulfonamides). The spreading of manure clearly increased the abundance of resistance genes in the soil. However, the diversity of tetracycline and sulphonamide resistance genes was high already prior to the manure application, although manure from intensive farming had not been applied in the previous years. This indicates that soils are a considerable pool of resistance genes. Whether this is the result of 50 years of tetracycline use or whether it is due to the natural background, these findings emphasize the role that environmental reservoirs might play in resistance gene capture.

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